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Allelopathic effects of *Paspalum notatum* Flüggé aqueous extract on seed germination and seedlings morphology of *Tagetes patula* L. and *Zinnia elegans* Jacq.

Viviane de Jesus do Carmo de Souza Pessoa[1], Rogério Gomes Pêgo[1]*, Rickson Gabriel dos Santos Farias[1]

ABSTRACT: The reuse of agricultural waste such as plant biomass enables the sustainable production of ornamental plants; however, these materials can contain allelochemical compounds that can affect the growth. This study aimed to investigate the allelopathic effects of aqueous extracts from Bahiagrass (*Paspalum notatum*) biomass on seed germination and seedling morphology of two ornamental species, Zinnia (*Zinnia elegans*) and French marigold (*Tagetes patula*). Five concentrations of aqueous extracts from Bahiagrass leaves were used to soak the seeds of each species that were conducted separately. The variables analyzed included germination counts, germination speed index, percentage of normal and abnormal seedlings, percentage of non-germinated seeds (dead and dormant), hypocotyl, root and total seedling length, and fresh seedling weight. The results showed that Bahiagrass aqueous extract do not affected the seed germination of Zinnia and French marigold, even at higher concentrations. However, the Bahiagrass extract caused deformities in Zinnia and French marigold seedlings, with higher concentrations leading to an increased percentage of abnormal seedlings in both species. These abnormalities included hypocotyl curvature, shortened or absent roots, and root oxidation.

Index terms: allelopathic compounds, Bahiagrass, French marigold, Zinnia.

RESUMO: A reutilização de resíduos agrícolas, como biomassa vegetal, permite a produção sustentável de plantas ornamentais; no entanto, esses materiais podem conter compostos aleloquímicos que podem afetar o crescimento. Este estudo objetivou investigar os efeitos alelopáticos de extratos aquosos da biomassa de grama Bahia (*Paspalum notatum*) sobre a germinação e a morfologia de plântulas de duas espécies ornamentais, Zínia (*Zinnia elegans*) e cravo-de-defunto (*Tagetes patula*). Cinco concentrações de extratos aquosos de folhas de grama Bahia foram utilizadas na embebição de sementes de cada espécie, conduzidas separadamente. Foram avaliadas a contagem de germinação, índice de velocidade de germinação, porcentagem de plântulas normais e anormais, porcentagem de sementes não germinadas (mortas e dormentes), comprimento do hipocótilo, da raiz e total das plântulas e peso fresco. Os resultados mostraram que o extrato aquoso de grama Bahia não afetou a germinação de sementes de zínia e tagetes defunto, mesmo em concentrações mais elevadas. No entanto, o extrato de grama Bahia causou deformidades em plântulas de Zínia e cravo-de-defunto, com concentrações mais elevadas levando a um aumento na porcentagem de mudas anormais em ambas as espécies. Essas anormalidades incluíram curvatura do hipocótilo, raízes encurtadas ou ausentes e oxidação radicular.

Termos para indexação: compostos alelopáticos, Grama Bahia, Cravo-de-defunto, Zínia.

***Corresponding author**
engagropego@yahoo.com.br

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[1] Programa de Pós-Graduação em Fitotecnia da Universidade Federal Rural do Rio de Janeiro, Seropédica, Rio de Janeiro, Brasil.

INTRODUCTION

Edible flowers have been consumed as human food for hundreds of years in different parts of the world, mainly due to their pharmacological properties (Mlcek and Rop, 2011; Zhao et al., 2019).

This is the case for Zinnias (*Zinnia elegans* Jacq.) and French marigolds (*Tagetes patula* L.), both of the Asteraceae family, are commonly cultivate as ornamental plants to decorative propose and also edible flowers utilized for garnishing dishes or in the preparation of teas (Pêgo et al., 2022; Khalid et al., 2024).

To cultivate edible flowers, it is suggested to adopt sustainable techniques, preferably organic crops (Santos and Reis, 2021; Santos et al., 2022). An innovative technique for organic vegetable production is the greenponics, which consists of using grass clippings of Bahiagrass (*Paspalum notatum* Flüggé) as non-composted biomass as a substrate to growing plants (Souza et al., 2024a). Greenponics has shown excellent results for production of pepper, cherry tomato and kale, achieving production results similar to traditional organic crops (Gentile et al., 2020; Souza et al., 2022; Souza et al., 2024b).

Despite the advantages of greenponics for the sustainable cultivation of some plant species, some challenges associated with the use of Bahiagrass straw were highlighted in short-cycle crops, such as lettuce, such as the stagnation of the initial growth of seedlings possibly caused by the presence of allelochemical components (Souza et al., 2024b).

Studies indicate that several grasses produce allelopathic compounds, that is, secondary metabolites that, when released into the environment, influence the emergence and development of neighboring plants (Favaretto et al., 2018). In the Poaceae's family the main allelochemicals identified are phenolic acids, hydroxamic acids, alkaloids and quinones (Sánchez-Moreiras et al., 2003). Within the *Paspalum* genus, research shows that allelochemicals produced by different parts of the plant directly affect the germination and early growth of several species (Zaman et al., 2018; Hassan and Mohamed, 2020). In ornamental production, allelopathic extracts can cause reduction in productivity and aesthetic value (Pêgo and Fialho, 2018; Hu et al., 2019; Chen et al., 2020).

Considering the production under the *greenponics* technique of two ornamental and edible flowers, Zinnia and French marigold, propagated by seed, as well as previous reports on the presence of allelopathic compounds in the biomass of grasses, the hypothesis was raised that these species may exhibit different sensitivities to the allelochemicals present in Bahiagrass. Therefore, the aim of this study was to evaluate the allelopathic potential of aqueous extracts from Bahiagrass on the seed germination and morphology of Zinnia and French marigold seedlings.

MATERIAL AND METHODS

Bahiagrass (*P. notatum*) leaves were manually harvested from gardens around Agronomy Institute of *Universidade Federal do Rio de Janeiro*, Seropédica campus, RJ. The leaves were dried in a forced air circulation oven at 65 °C for 72 hours and then ground to obtain a homogeneous powder.

To prepare the aqueous extract, the proportion of 12 g of dry grass per 100 mL of distilled water, equivalent to 12% (Weight/Volume) was considered. The dry leaf biomass was infused in distilled water at room temperature (25 °C) for 24 hours and then filtered using hydrophilic cotton and then in a 180µM thick paper filter to obtain the crude extract of Bahiagrass.

The crude aqueous extract was established as a solution composed of 100% of compounds extracted. The treatments of the experiment were obtained by dilution of crude extract and distillated water to obtain the following treatments: 100% (crude extract), diluted extract to 75%, 50% and 25%. The control was established as 0% (distilled water only).

To verify the allelopathic effectiveness of the crude extract of Bahiagrass, lettuce (*Lactuca sativa*) seeds were used as target plant due to its high sensitivity to different allelochemical compounds according Amâncio et al. (2021). Previously testing sensitive plants exposing them to allelopathic extracts is important to certify the presence of compounds before using on other plant species that can be tolerant. Specifically for lettuce, only the concentrations of the crude extract (100%) and the control composed of distilled water (0%) were tested.

For Zinnia and French marigold seeds all extract concentrations were tested. To each species, 50 seeds were placed in a 120 x 20 mm Petri dish lined with two Whatman 150 mm filter paper moistened with 5 mL of different concentrations of aqueous extracts. Treatment control consisted of moistening paper with distillated water only (0%). Independent experiments were performed for each species. For each treatment, four replicates were used and maintained in a completely randomized design (CRD). The seeds were incubated in a growth chamber at 20 °C, according to Rules for Seed Testing (Brasil, 2009).

Seed germination was evaluated daily, and the Germination Speed Index (GSI) was calculated by the formula proposed by Maguire (1962):

$$GSI = \frac{N_1}{D_1} + \frac{N_2}{D_2} + \frac{N_3}{D_3} + \cdots + \frac{N_n}{D_n}$$

where N_i is the number of germinated seeds at time D_i.

The first count (vigor) and the final count of germination percentage were obtained at the fourth and seventh days from the beginning of the test, respectively (Brasil, 2009). It was considered germinated the seeds that had radicles greater than 0.5 cm in length. On the seventh day after the start of the test, the percentage of normal and abnormal seedlings was evaluated. Seedlings that presented well-developed roots and aerial parts, absence of visible damage or necrosis of the tissues, and without pronounced curvatures of hypocotyls and roots, were considered normal. Immediately after, the roots length, hypocotyl length, total length and the fresh weight of seedlings were also measured using four replicates of ten seedlings per treatment. To measure aerial and root parts a digital caliper was used, and to measure the fresh weight, an analytical balance with 0.0001 g of precision was used.

Data normality was verified using the Shapiro-Wilk test and, when necessary, data were transformed. For the variables first and second count and GSI, data were transformed to . Data were subjected to analysis of variance (ANOVA) at 5% probability and, when significant, were subjected to regression analysis to determine the values of the correlation coefficient (r), selecting the models with the best fit. The SISVAR statistical software was used in all the analyses.

RESULTS

Confirming the presence of allelopathic compounds in Bahiagrass aqueous extracts, it was observed that vigor, germination and seedlings performance of lettuce were significantly affected.

Lettuce seeds (indicator species) had their germination percentage reduced from 98.0% to 71.5% in the presence of the extract, in the analysis carried out on the fourth day after the start of the test, a reduction of 26% (Table 1). In addition, there was a significant reduction in the percentage of normal seedlings and a proportional increase in percentage of abnormal seedlings on the seventh day from the start of the germination test (second count).

Table 1. First count (FC) and second count (SC) of seed germination, normal seedlings (NS), abnormal seedlings (AS), hypocotyl length (HL), root length (RL), total length (TL) and fresh weight (FW) of lettuce seedlings of the Regina variety treated or not with aqueous extracts of Bahiagrass.

Treatment	FC (%)	SC (%)	NS (%)	AS (%)	HL (cm)	RL (cm)	TL (cm)	FW (mg)
Control	98.0*	100.0^{ns}	95.0*	5.0*	6.4*	6.3*	12.7*	28.1*
Extract 100%	71.5	95.5	5.5	86.5	3.3	1.3	4.6	15.2
CV (%)	10.6	10.9	13.9	23.8	4.8	10	8.3	11.1

*Means differ significantly; ^{ns}means do not differ significantly by F-test (5% probability). CV: coefficient of variation.

When germinated in the presence of Bahiagrass extract, lettuce seedlings suffered reductions in hypocotyl length (HL), root length (RL), total length (TL) and fresh weight (FW) by 48.4%, 79.4%, 63% and 45.9% respectively (Table 1).

The main abnormalities observed in lettuce seedlings were the presence of root necrosis, curvatures and chlorosis of hypocotyls and roots, short roots and absence of aerial parts (Figure 1).

There was no significant difference between the concentrations of the aqueous extracts of Bahiagrass in the germination of Zinnia seeds; the germination counts on the fourth and seventh day were 61.5% and 71.3% respectively (Table 2). Similarly, the extract concentration did not affect the germination of French marigold seeds, whose germination percentage on the fourth and seventh days were 78.5% and 82.8% respectively (Table 3).

The extracts did not significantly affect the dormant and dead seeds percentage for Zinnia, which reached average percentages of 9.1% and 19.9%, respectively (Table 2). Similarly occurred to French marigold, in which was possible to observe 17.2% and 0% of dormant and dead seeds respectively (Table 3).

The germination speed index was also not affected by the different treatments, allowing obtaining average values of 41.8 and 59.2 for Zinnia and French marigold respectively (Table 2 and 3).

The maximum occurrence of normal seedlings for French marigold was 81.3% in the control treatment (0% grass extract), but in the higher concentration of the extract only 5.5% of normal seedlings were observed (Figure 2A). For Zinnia, there was a reduction in normal seedlings from 60.2% to 32.4%, equivalent to 46.2% (Figure 2A).

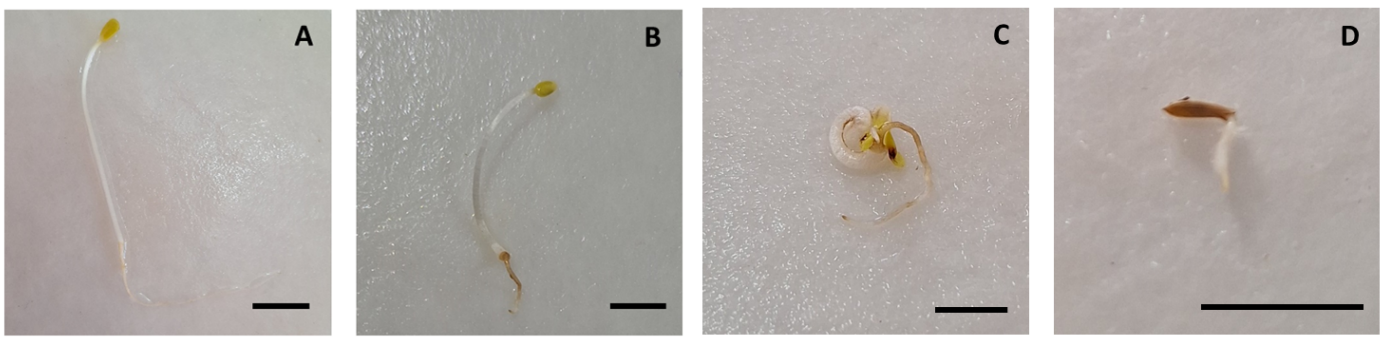

Figure 1. Morphology of lettuce. Normal seedling (A); abnormal seedling showing chlorotic and short roots (B); curvature and chlorosis in the hypocotyl and roots (C) and absence of aerial parts and short roots (D). The bars are equivalent to 1 cm in length.

Table 2. First count (FC) and second count (SC) of seed germination, dormant seeds (DmS), dead seeds (DS) and germination speed index (GSI) of Zinnia seeds treated in different concentrations of aqueous extracts of Bahiagrass.

Concentration	FC (%)	SC (%)	DmS (%)	DS (%)	GSI (index)
0%	61.5 ± 0.96	73.5 ± 1.50	12.5 ± 3.10	14.0 ± 2.00	44.0 ± 1.13
25%	67.0 ± 2.38	74.0 ± 2.83	10.0 ± 1.29	17.0 ± 1.29	47.2 ± 2.19
50%	54.5 ± 5.06	65.5 ± 2.63	12.0 ± 2.45	23.0 ± 2.37	37.4 ± 2.37
75%	64.0 ± 4.97	73.0 ± 4.04	5.0 ± 3.00	22.0 ± 3.32	42.8 ± 3.32
100%	60.5 ± 1.50	70.5 ± 2.22	6.0 ± 1.50	23.5 ± 2.34	37.6 ± 2.34
Mean	61.5	71.3	9.1	19.9	41.8

Table 3. First count (FC) and second count (SC) of seed germination, dormant seeds (DmS), dead seeds (DS) and germination speed index (GSI) of French marigold seeds treated in different concentrations of aqueous extracts of Bahiagrass.

Concentration	FC (%)	SC (%)	DmS (%)	DS (%)	GSI (index)
0%	82.0 ± 3.30	85.5 ± 3.10	14.5 ± 3.30	0	64.3 ± 1.7
25%	73.0 ± 3.46	82.0 ± 3.46	18.0 ± 3.46	0	57.8 ± 2.76
50%	81.5 ± 4.32	84.0 ± 4.32	16.5 ± 4.32	0	60.3 ± 3.14
75%	82.0 ± 2.87	86.5 ± 2.87	13.5 ± 2.87	0	60.5 ± 1.61
100%	72.0 ± 5.83	76.0 ± 2.99	24.0 ± 2.99	0	52.9 ± 5.04
Mean	78.1	82.8	17.2	0	59.1

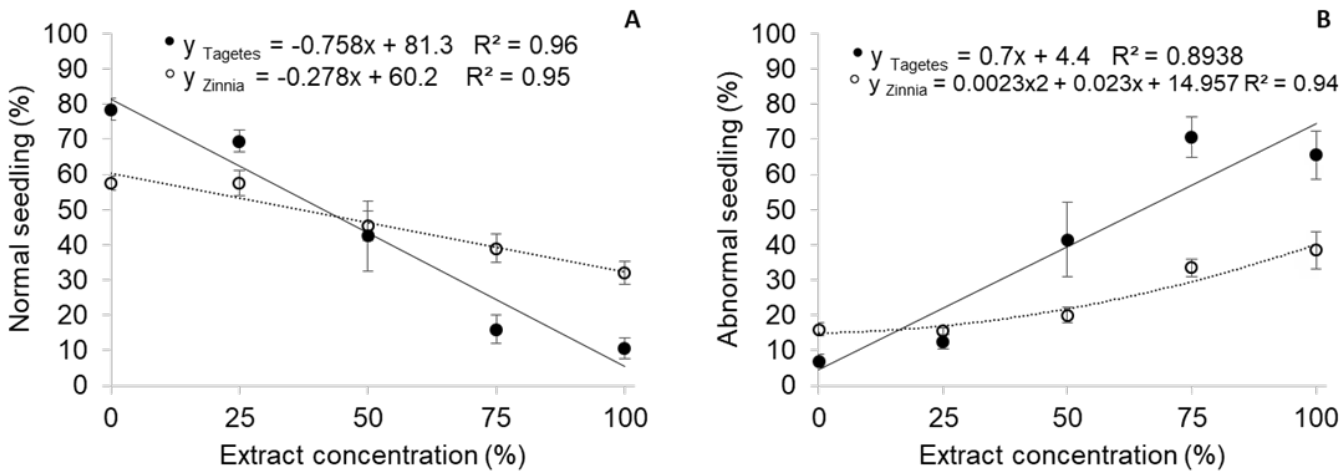

Figure 2. Percentage of normal (A) and abnormal (B) Zinnia and French marigold seedlings treated with different concentrations of Bahiagrass aqueous extracts.

A significant increase of abnormal seedlings in zinnia and French marigold could be observed proportionally to the increase in the concentration of Bahiagrass extract, up to 74.4% for French marigold and 40.3% for Zinnia, an increase of 70% and 25.4% respectively (Figure 2B).

The most frequently observed abnormalities in Zinnia and French marigold seedlings consisted of chlorosis in roots or hypocotyls, hypocotyl curvatures, presence of short roots and prominent chlorosis, likely as high seed without aerial parts and necrotic roots production (Figure 3).

Hypocotyl, root and total length of Zinnia and French marigold seedlings were significantly affected by the gradual increase in concentration of Bahiagrass extract.

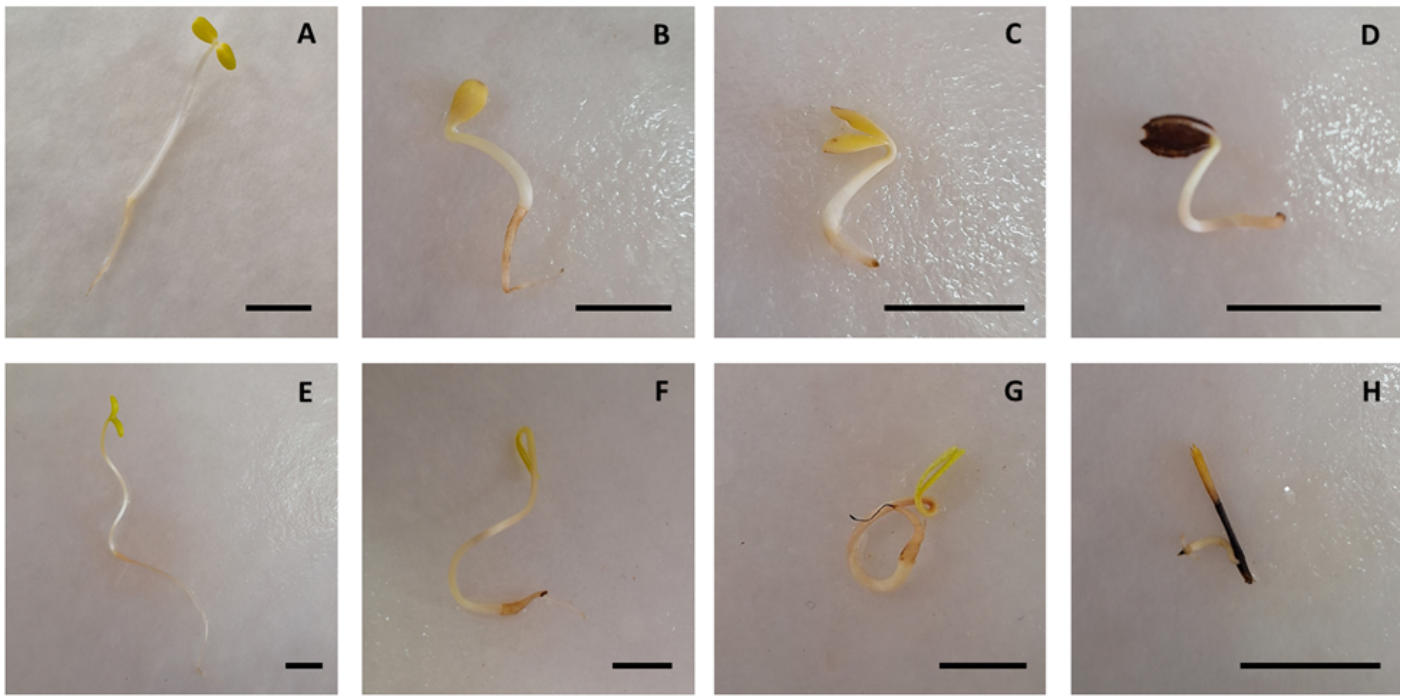

Figure 3. Visual appearance of Zinnia and French marigold. Normal plant of Zinnia (A) and French marigold (E), abnormal seedling showing browning of Zinnia (B) and French marigold (F); necrosis of roots of and curvature of hypocotyl of Zinnia (C) and French marigold (G) and absence of aerial parts of Zinnia (D) and French marigold (H). The bars are equivalent to 1 cm in length.

The hypocotyl length of Zinnia and French marigold seedlings from control (0%) were 3.2 cm and 4.2 cm respectively. However, when the seeds were treated with the highest concentration of Bahiagrass extract, the average hypocotyl length of the seedlings was 1.5 and 1.3 cm, respectively, a reduction equivalent to 53.1% and 69.0% (Figure 4A).

The root length of Zinnia and French marigold seedlings control (0%) were 3.5 cm and 4.0 cm, respectively; those germinated at the highest concentration extract reached only 1.6 and 0.5 cm, respectively, representing a reduction of 54.3 and 87.5% in root length (Figure 4B).

Zinnia and French marigold seedlings showed their greatest growth in length when moistened with distilled water only (control), reaching values of 6.7 and 8.2 cm, respectively, and reaching the smallest lengths when treated with Bahiagrass crude extract, of 3.2 and 1.8 cm respectively (Figure 4C). This represents a reduction of 52.2 and 78.0% in total length. Similarly, the increase in the concentration of the Bahiagrass extract also affected the fresh weight of Zinnia and French marigold seedlings, with the highest values seen at control (0%), 0.645 and 0.273 g respectively; lowest values achieved were 0.345 and 0.123 g, respectively, at the highest concentration, representing a reduction of 46.5 and 54.9% in fresh weight (Figure 4D).

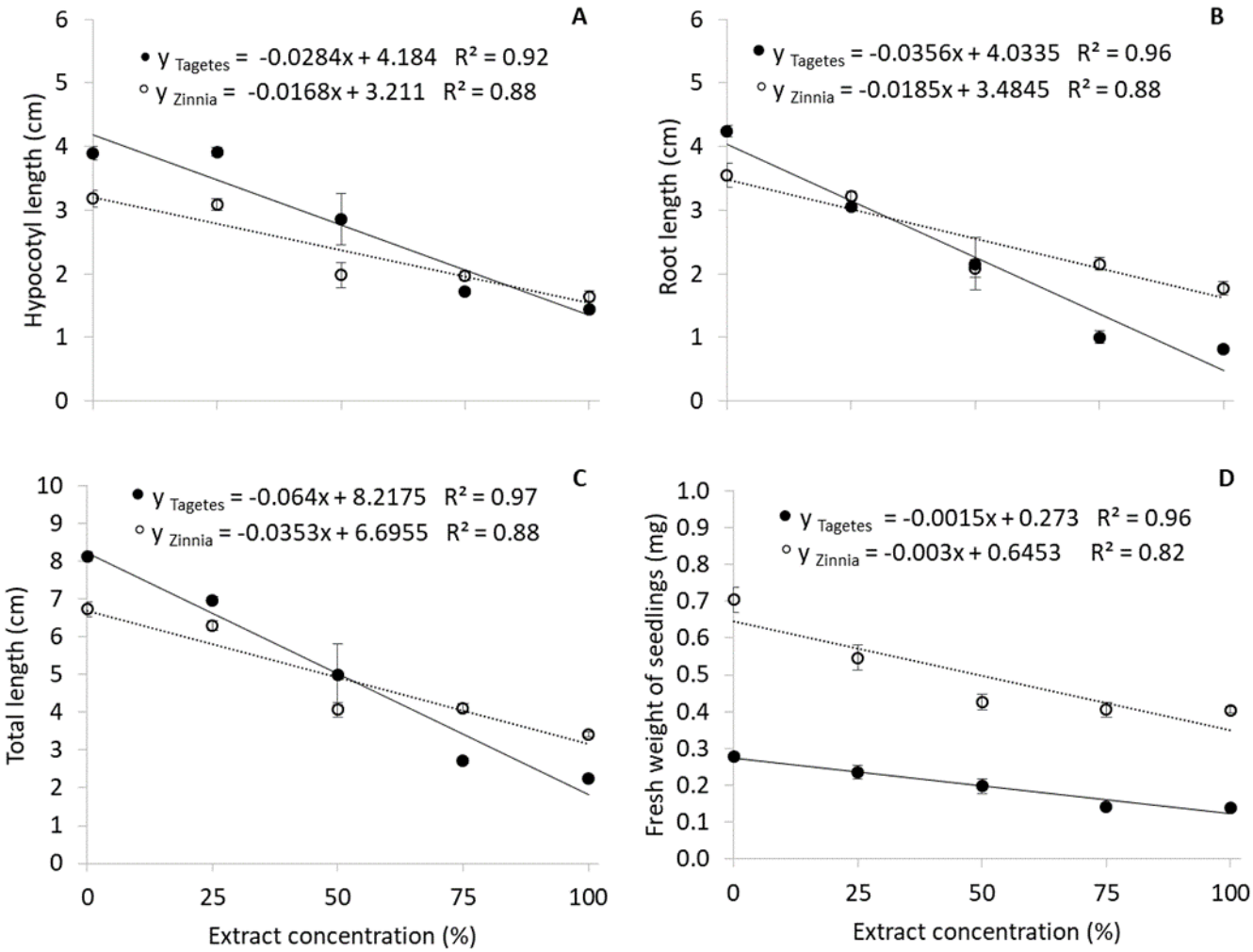

Figure 4. Hypocotyl length (A), root length (B), total length (C) and fresh weight (D) of Zinnia and French marigold seedlings grown in different concentrations of Bahiagrass aqueous extracts.

DISCUSSION

Lettuce (*Lactuca sativa*) is frequently used as a target plant in allelopathy studies, mainly due to its sensitivity even at low concentrations of allelochemicals (Souza et al., 2007; Reigosa et al., 2013; Favaretto et al., 2018; Rezendes et al., 2020; Hammami et al., 2025). The treatment of lettuce seeds with crude extracts (100%) of Bahiagrass indicated a significant reduction in the expression of vigor, determined by the first germination count (four days after the start of the germination test). However, no effect on the germination parameters was observed for Zinnia and French marigold plants, regardless of the extract concentration tested, indicating that these species may have different levels of sensitivity to chemical compounds in the Bahiagrass aqueous extract.

Abnormality in seedlings is one of the main effects of allelopathy in plant species. These abnormalities can be seen by the deformity or curvature of hypocotyls, thickening or shortening of roots and by the phytotoxicity of these organs (chlorosis and necrosis) (Caser et al., 2020; Bergamin et al., 2024), like the ones observed in this study.

Recent research on ornamental plants indicates that allelopathic compounds can affect their development by different forms. Seedling abnormality is one of the main effects caused in ornamental plants, especially on hypocotyl and root length (Pêgo and Fialho, 2018). Xie and Wang (2024) reported that ornamental plants *Ophiopogon japonicus*, *Senecio scandens* and *Viola philippica* also had their hypocotyl and root lengths reduced by allelochemicals, which

was caused by physiological stress observed by increased levels of catalases and soluble proteins in the plant. These differences are related to variations in absorption mechanisms, translocation, and site of action of substances among different species (Choudhary et al., 2023).

Hu et al. (2019) reported the allelopathic effects of the grass *Poa pratensis* on Zinnia and French marigold, which suffered a reduction in germination percentage, hypocotyl and root length, like the effects observed in this study.

Specifically for species from Poaceae family a great diversity of allelochemicals were isolated, mostly represented by phenolic acids, alkaloids, flavonoids, hydroxamic acids, terpenes and quinones (Sánchez-Moreiras et al., 2003; Favaretto et al., 2018). Shoot extracts from *Paspalum maritimum* had secondary metabolites as condensed tannins, chalcones and aurones, flavanones, steroids and saponins responsible for inhibition of seed germination and seedling development (Pereira et al., 2019).

The presence of allelochemicals in *Paspalum dilatatum* had negative effect on growing ornamental species in urban gardens; the effectiveness of these compounds in reducing plant growth varies between plant species (Hassan and Mohamed, 2020).

This research shows important contributions to the effect of Bahiagrass on ornamental plants, as well as lettuce. In the search for alternative uses of sustainable resources, grass can be used as cover for cultivation beds and as a source of substrate for plant production. However, it is worth mentioning that seedlings of species sensitive to allelopathic compounds can be negatively impacted by the presence of grass straw in the early stages of their development.

CONCLUSIONS

This study shows that increasing the concentration of Bahiagrass (*Paspalum notatum*) extract does not affect seed germination percentages but may cause anomalies or restrict the early growth of Zinnia (*Zinnia elegans*) and French marigold (*Tagetes patula*) seedlings, regardless of its concentration.

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AUTHORS' CONTRIBUTION

Viviane de Jesus do Carmo de Souza Pessoa contributed with: Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Visualization, Writing - original draft; Rogério Gomes Pêgo contributed with: Conceptualization, Formal analysis, Data curation, Visualization, Writing—review & editing; Rickson Gabriel dos Santos Farias contributed with: Visualization, Writing—review & editing.

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